OBSERVATIONS ON THE SPERMATOGENESIS OF THE GALL-FLY, DRYOPHANTA ERINACEI (MAYR).

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INTRODUCTION.

Dryophanta erinacei is one of the gall-producing *Hymenoptera* having two generations in the year: males and females in the spring, and females alone in the fall. The fertilized eggs of the bisexual generation produce females exclusively, while the unfertilized eggs of the female generation produce both males and females.

The material for this study was obtained on April 13, 1914, from galls occurring on white oak trees (*Quercus alba*) in the neighborhood of Cincinnati. The galls are smooth spherical protuberances on the bud-scales, several millimeters in diameter. Usually one, but occasionally two, and sometimes three galls very close together are found at the end of a single twig. Twigs bearing galls were cut off, brought into the laboratory, and the cut ends inserted in sand moistened with water. Ten days later males and females emerged from the galls, and continued emerging for two weeks. Copulation took place immediately after emergence.

These galls supplied all stages of developing males and females from the late larva to the imago. For fixation Petrunkewitsch's fluid was used; penetration being facilitated by making a longitudinal incision through the body wall. In some cases ovaries and testes were dissected out, but better results were obtained when the organs were left in situ and the entire animal sectioned.[1]

Embedding was done in rubber-paraffin, and serial sections cut 10 μ in thickness. Two methods of staining were used: safranin and light-green, and iron-haematoxylin with or without counterstain. After dehydration the stained sections were cleared and

[1] For assistance in collecting and preserving material I wish to acknowledge my indebtedness to Dr. Annette F. Braun.

mounted in euparal. Euparal offers several advantages over balsam as a mounting medium. Thus its use obviates running stained sections through absolute alcohol; since sections may be transferred directly from 95 per cent. alcohol to euparal. Next the index of refraction of euparal is low 1.483. And lastly euparal dries quickly, so that sections may be studied at the end of twelve to twenty-four hours after mounting, without danger of injury.

Observations.

The testes of the late larva and early pupa show primary spermatocytes at the end of the growth period as large polygonal cells having a reticulated nucleus containing a poorly defined nucleolus, often of a bipartite character (Fig. 1). The nucleolus does not take the safranin stain as deeply as the chromosomes, and thus differs markedly from the chromosome nucleolus of the primary spermatocytes of many *Hemiptera*.

A true primary spermatocyte division does not occur. Instead, a small mass of cytoplasm free of chromatin is constricted off, forming the so-called polar body. Preparation for this suppressed or abortive division begins with a change in the outline of the cell, the spermatocyte assuming a pear shape (Figs. 2, 3 and 4). From the narrow end of the cell and forming the stem of the pear, extends a short filar process. At the base of this process, which at first glance suggests the tail of a spermatozoon, is often found a light basic-staining spherule which may or may not be a centrosome. While these changes are taking place in the cytoplasm the nucleus undergoes a slight contraction and the chromatin passes through a series of transformations terminating in the formation of chromosomes (Fig. 4).

The next step in the process is somewhat uncertain and there may be some question as to seriation. It seems that after the chromosomes are completely formed, they become massed in clumps at one side of the nucleus, and from these masses distinct loops extend toward the opposite side of the nucleus (Figs. 5 and 6). The cell shortens, the filar process becomes less distinct (Fig. 6), and a portion of the cytoplasm is constricted off (Figs. 7 and 8). As this is taking place the nuclear membrane appears very irregular in outline but seems to remain intact. Inside the

nuclear area the chromosomes are in the form of single rods whose free ends extend toward the polar body. There is every appearance to indicate a resistance of the part of the chromosomes against a tension pulling toward the polar body. Distinct spindle fibers are not to be seen, but the cytoplasm contains a reticular structure which may represent a poorly developed spindle. The polar body is quickly cut off from the cell to which, however, it may remain attached for a considerable length of time (Fig. 15). The free polar body of Fig. 8 belongs to a cell in an adjacent section. Polar bodies cut in various planes are frequently seen in the spaces between spermatocytes at this time (Figs. 8 and 16) and throughout the second spermatocyte division. The complete absence of polar bodies in cysts containing cells with the chromosomes in the looped condition of Figs. 5 and 6 makes it almost certain that the looped stage precedes that of Figs. 7 and 8, in which the chromosomes show free ends.

Preparations for the second spermatocyte division follow very rapidly. After the formation of the polar body, the second spermatocyte rounds up; the knot of chromosomes separates into distinct, short, thick, curved rods, 12 in number (Fig. 9). In the cell figured here, a late prophase, the nuclear membrane is fairly distinct. Details of spindle formation were not observed. Figs. 10 and 11 show characteristic side-views of spindles at metaphase. The chromosomes seldom lie in one plane so that counting even in polar views is a difficult matter. In such views, as in Figs. 12 and 13, 12 chromosomes can be counted with considerable accuracy in the majority of cases.

A characteristic late telophase is shown in Fig. 14 which resembles to a striking degree a somatic mitosis, and strongly suggests that the chromosomes have been divided longitudinally. In later stages of this division (Fig. 15) the chromosomes become packed into dense compact masses, so that it is impossible to determine the number of constituent chromosomes in the daughter groups. When reconstitution of the nuclei occurs (Fig. 16), these masses break up into slightly bent rods of ragged outline. In cross section these rods appear as dots of which 12 can often be counted. Counts of the daughter groups of chromosomes made in this way are not very satisfactory, since one is

never sure that a cross-section includes all of the rods or that a single rod has not been cut more than once.

The spermatids formed by this division seem therefore to be equal in size and chromatin content, and all of them develop into spermatozoa. There is no evidence of a heterochromosome or chromatoid body passing undivided into one of the spermatids.

By the end of the second spermatocyte division all of the polar bodies are detached and show signs of disintegration, fragments being frequently seen in the intercellular spaces giving the appearance shown in Fig. 16.

The relatively distinct outline of the chromosomes seen in this last figure persists for but a short time and is completely lost in the young spermatids. Figs. 17 and 18 are early stages in the transformation of the spermatids into spermatozoa.

Such in brief is an outline of the main features of development of the germ cells in the male of *Dryophanta* from the growth period to the spermatids. There is but one true maturation division—that of the second spermatocyte. The first spermatocyte division is indicated by the pinching off of a small quantity of chromatin-free cytoplasm which forms the so-called polar body.

Discussion.

Doncaster in his studies of the gametogenesis of the gall-fly, *Neuroterus lenticularis*, arrived at certain conclusions which may be considered at this point. This species of *Hymenoptera* has a similar life-history to that of *Dryophanta*. Thus according to Doncaster the female generation emerges in April from galls formed during the preceding summer and immediately lays eggs in oak buds (species?). Early in summer the galls appear from which males and females emerge. After copulation the female lays eggs in the tissues of young leaves at the side of a small vein. From the galls resulting, females emerge in the following spring.

As in *Dryophanta*, therefore, the fertilized eggs of the bisexual generation develop into females; while the unfertilized eggs of the female generation produce both males and females.

Doncaster found that the first spermatocyte division is abortive—a small portion of the cytoplasm being constricted off as the polar body. This is followed by a resting stage which resembles

the metaphase of a true division, but is distinguished from it by the persisting nuclear membrane and the position of the chromosomes at one end of the nucleus near the broad end of the cell. No nuclear division takes place but the nucleus becomes oval in shape and the chromosomes generally contract to form a compact mass lying across its center. In some cells at least this chromatin mass seems to divide—one half passing to each side of the oval nucleus. The chromatin may finally disperse and give rise to a condition resembling the first spermatocyte in which the chromatin has begun to appear. "Possibly the division of the chromatin inside the nucleus, which occasionally seems to occur, is the persistent remnant of a true nuclear division, or it may be compared with the 'intranuclear karyokinesis' described by Kostanecki in the parthenogenetic eggs of *Mactra*" (p. 93). Toward the end of the rest stage the chromatin becomes grouped in the form of large elongate granules or small bands having a more or less meridional arrangement under the membrane.

The second spermatocyte division in *Neuroterus* is a true mitotic division in which the haploid number of chromosomes, 10, appears on the spindle to be equally divided between the daughter cells. There is also a small stained body lying outside of the spindle which passes undivided to one of the spermatids.

In the spermatogonia and in mitotic figures of nerve cells in the developing nervous system Doncaster finds the halpoid number of chromosomes, 10, but in mitoses of immigrant mesoderm cells the diploid number, 20.

The eggs layed by the females of the bisexual generation undergo two maturation divisions; leaving 10 chromosomes for the female pronucleus. The spermatozoon brings into the egg 10 chromosomes, and 20 chromosomes appear on the cleavage spindles. The parthenogenetic eggs of the female generation may be divided into two groups: Those which undergo maturation and develop into males; those which omit the maturation divisions and develop into females. In the first group 10 chromosomes are found in the cleavage divisions; in the second group 20. Since any female produces only one kind of egg, there are male-producing females and female-producing females.

Mitoses in the nervous system of all females show the diploid number of chromosomes.

Returning now to *Dryophanta* I should like first to consider the stage represented in Figs. 5 and 6, which I believe corresponds to the second spermatocyte resting stage mentioned by Doncaster in *Neuroterus*. The figures at first glance suggest the synapsis stage of other insects, but in view of other facts it is difficult to interpret the condition as a fusion of chromosomes. Earlier stages such as the prophase shown in Fig. 4 display the same number of chromosomes as appears in the second spermatocyte division, 12, which is assumed to be the haploid number approximately. Since there is no evidence in *Dryophanta* of an intra-nuclear division of these 12 chromosomes into two groups, a true synapsis at this time would be equivalent to a second "reduction." A more probable interpretation of this "looped stage" and one that is warranted by a close study of the sections is that the limbs of a loop are the halves of a chromosome that has undergone a temporary and incomplete splitting. With the next step in the process, the formation of the polar body, the split disappears and the chromosomes have every appearance of being single, solid rods (Figs. 7 and 8). The latter condition might of course be brought about by breaking of the loops at the middle, but in that event one would expect to find twice as many single chromosomes as loops. Such is not the case, for the number of unsplit chromosomes is the same as the number of loops so far as could be determined. Reversing the seriation at this point would of course change the interpretation offered here; but the main reason for placing the looped stage before the other, as has been mentioned above, is that there is no evidence of polar body formation at this time. And to this may be added the fact that the outline of the cell at the looped stage as shown in Fig. 6 represents an intermediate condition between that of Fig. 4 in which there can be no question about polar bodies being absent, and Figs. 7 and 8, in which the polar bodies certainly are present.

An actual resting stage, if one occurs at this time, must be of very short duration. The second spermatocyte division follows very quickly after the formation of the polar body. Fig. 9 represents a prophase of this division in which the chromosomes are surrounded by an intact nuclear membrane. The spindle

area of the second spermatocyte is rather distinctly marked off from the rest of the cytoplasm (Fig. 10) and suggests that the nuclear membrane disappears very slowly.

Polar views of the metaphase display, as nearly as could be determined, 12 chromosomes, presumably the haploid number. It would seem that each chromosome is divided quantitatively by a longitudinal splitting; although it must be remembered that attempts at verifying this conclusion by studying the constituents of the daughter groups are not satisfactory owing to the tangled condition of the chromosomes.

I find nothing resembling the small stained body which in *Neuroterus* according to Doncaster passes undivided to one of the spermatids. As Wilson has observed this body is of the same nature as the chromatoid body seen in the growth-period and spermatocyte-division of *Pentatoma*. The chromatoid body is of rounded form, dense and homogeneous consistency, and after double staining with haematoxylin or safranin and light green is at every stage colored intensely blue-black or brilliant red, precisely like the chromosomes of the division period or the chromosome-nucleoli of the growth period. Nevertheless Wilson finds that the body is neither a chromosome nor any kind of a chromosome and takes no visible part in the formation of the spermatozoa. In the transformation of the spermatids it wanders far into the sperm-tail and is at last cast off altogether.

I have not yet had opportunity to study the maturation phenomena of the egg in either generation of *Dryophanta*, but observations confined to individuals of the bisexual generation point to general conclusions which differ somewhat from Doncaster's views regarding the chromosomal relations in the alternate generations. In the material at my disposal spermatogonial divisions are not abundant enough to determine the number of chromsomes. While mitoses abound in the somatic cells of male larvae and pupae, it is difficult to find good clear metaphases; but wherever counts were possible, the number found was 12 (Fig. 19). In the follicle cells of the ovary I have found it less difficult to count the chromosomes. Figs. 20, 21 and 22 are drawings of metaphase plates of such cells in which the numbers are respectively 13, 14 and 13.

In the somatic cells of both males and females one occasionally finds mitotic figures containing a much larger number of chromosomes, but such cases are in the nature of exceptions and no one would contend that they represent an average condition. If there is such a thing as constancy in the number of chromosomes in the majority of somatic cells, the constant is in the neighborhood of 12 in both males and females of the bisexual generation. Because this is the number of chromosomes found in the second spermatocyte division, 12 is assumed to be the approximate haploid number. Now in any case where an accurate count is difficult or impossible in the somatic cells, it is always possible to determine with certainty that the number is very much less than the expected diploid number 24. In view of the fact, that in the honey-bee it is said that the somatic mitoses show a very much higher number of chromosomes than occurs in the gonial cells, somatic mitoses should not be used as a safe and reliable method of determining the diploid number. There may however be some significance in the fact that a large number of somatic cells of both males and females of *Dryophanta* contain a number of chromosomes that approximates the number found in the dividing spermatocyte rather than a multiple of this number.

Any definite statement regarding the origin and significance of this condition must await examination of the maturation and cleavage spindles of the egg. However, the facts at hand do suggest that the males and females of the bisexual generation of *Dryophanta* develop from eggs whose chromosomes have undergone reduction in maturation. The slightly large number of chromosomes found in the females somatic tissues may or may not be of significance, but if sex determination has its basis in the chromosomes, a difference in the method of distribution of the chromosomes in maturation may explain why some of these eggs develop parthenogenetically into females and others into males.

In a recent paper Nachsheim has summed up in a general statement the results of investigations dealing with sex-determination in *Hymenoptera* as follows: "Die Männchen der Hymenopteren entstehen aus unbefruchtete Eiern, die zwei

Richtungskörper abgeschnürt und eine Reduktion ihrer Chromosomenzahl erfahren haben. Sie besitzen also nur ein Chromosomensortiment, das mütterliche, und infolgedessen muss in der Spermatogenese die Reduktionsteilung unterbleiben. Die Weibchen der Hymenopteren besitzen beide Chromosomensortimente, also die diploide Chromosomenzahl in ihren somatischen Zellen, da sie aus befruchteten Eiern ihre Entstehung nehmen oder—bei den Blatt- und Gallwespen—zwar ebenfalls aus unbefruchteten Eiern, aber aus solchen, die den Reifungsteilungen ihre Chromosomenzahl nicht reduciert haben; entweder findet in diesen Eiern überhaupt nur eine Reifungsteilung statt, oder beide Reifungsteilungen sind Aquationsteilungen. Der zweite Richtungskörper kann also. . . . an Stelle der Spermatozoons treten, d.h. der zweite Richtungskörper bringt in Verbindung mit der Eikern dasselbe Geschlecht hervor wie der Eikern in Verbindung mit einem Spermakern" (pp. 220–221).

My findings in the somatic chromosomes of *Dryophanta* raises the question as to whether females of the bisexual generation are produced parthenogenetically from eggs that do not undergo reduction in maturation. An examination of maturation stages in the egg is necessary to decide this point and material for this purpose is being collected at the present time.

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EXPLANATION OF PLATES.

The figures are camera drawings made at table level with Zeiss apochromatic objective, 1.5 mm. and compensating ocular, 12. There has been some reduction in reproduction.

PLATE I.

FIG. 1. Primary spermatocyte at the end of the growth period. Male pupa.

FIGS. 2, 3 AND 4. Primary spermatocytes undergoing changes in outline preliminary to the formation of the polar body.

FIGS. 5 AND 6. Primary spermatocytes having chromosomes in the form of loops or split rods.

FIGS. 7 AND 8. Stages in the cutting off of the polar body. Fig. 8 contains a second polar body belonging to a cell in a neighboring section.

FIG. 9. Prophase of the second spermatocyte division showing 12 chromosomes.

FIG. 10. Side view of the second spermatocyte spindle at metaphase.

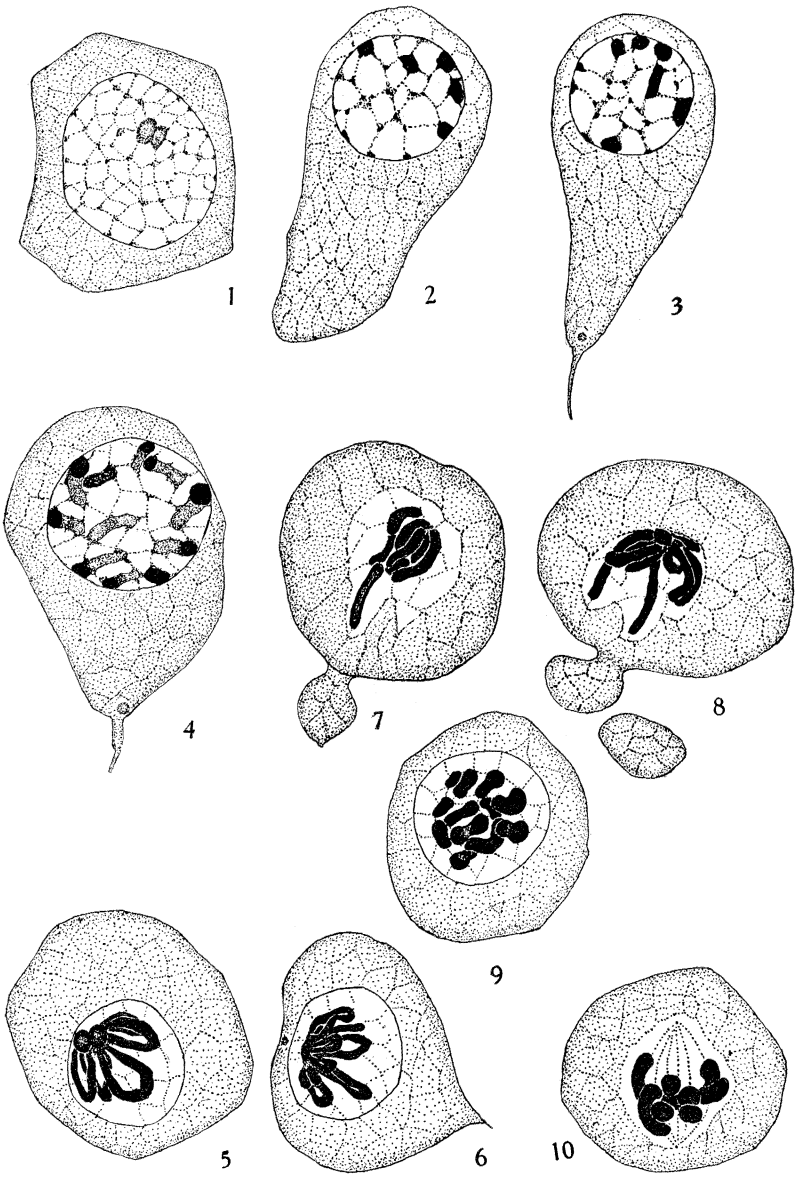

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PLATE II.

FIG. 11. Side view of the second spermatocyte spindle at metaphase.

FIGS. 12 AND 13. Polar views of the second spermatocyte spindle at metaphase showing 12 chromosomes.

FIG. 14. Second spermatocyte spindle at late anaphase showing a free polar body near the upper end of the cell.

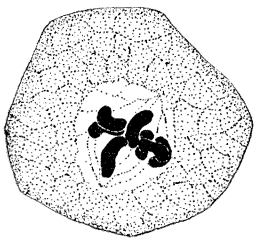
FIG. 15. Second spermatocyte at telophase with a polar body attached to the upper daughter cell.

FIG. 16. Early spermatid, reconstruction of the nuclei. Polar body fragments near the upper cell.

FIGS. 17 AND 18. Stage in the transformation of spermatids into spermatozoa.

FIG. 19. Metaphase chromosome group in the mitosis of a developing wing, showing 12 chromosomes. Young male pupa.

FIGS. 20, 21 AND 22. Metaphase chromosome groups of ovarian follicle cells, showing 13, 14 and 13 chromosomes respectively. Late female larva.



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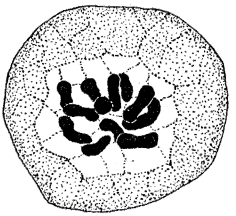

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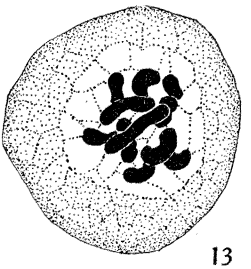

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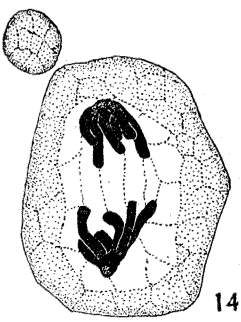

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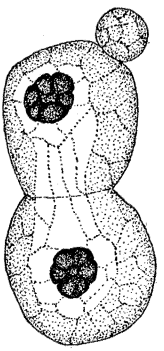

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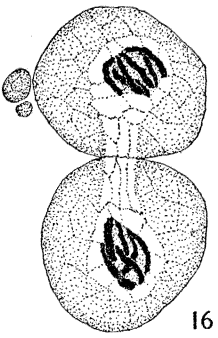

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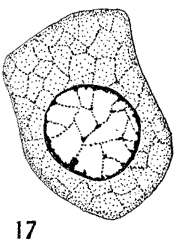

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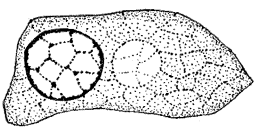

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